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EFFECT OF GAMMA-RADIATION FROM COBALT-60 ON THE PROCESS OF OVULATION, FERTILIZATION, AND EMBRYONIC DEVELOPMENT OF A FROG

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ABSTRACT: The paper discusses the effect of penetrating radiation from cobalt-60 on the ovulation, fertilization, and embryonic development of a frog. Particular significance is given to the role of the pituitary gland of the mother organism. It is established that the embryonic development of a frog is particularly affected by irradiation both of the females and of the males.

The methods of artificial inducement of ovulation and artific- /389* ial fertilization developed by Kashchenko at the Nemilova Laboratory were used in this study.

Concerning the frogs under investigation, the ovules of the females are in the ovaries during the winter months, and are not ready for fertilization, while the males have mature spermatozoids. These frogs were subjected to irradiation by γ-rays. The source of radiation was cobalt-60. The frogs were given a sublethal dose of 6800-7500 g during a 48-hour exposure.

In Series I of the experiments, a suspension of pituitrin was injected into the abdominal cavity of the females on the day after the end of the irradiation; the purpose was to induce ovulation. In one case, an irradiated female frog was administered a suspension of powdered pituitrin taken from two other irradiated females. In another case, an irradiated female was administered a suspension of pituitrin taken from nonirradiated females. In the third case, an unexposed, healthy female was injected with a suspension of pituitrin taken from irradiated females. Finally, in the fourth case, which was the control, an unexposed female was administered a suspension of pituitrin taken from nonirradiated females.

After 2 days, the injections with pituitrin were repeated in the same combinations. After another two days, the abdominal cavity was dissectioned, and the results of the experiments were recorded.

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* Numbers in the margin indicate pagination in the foreign text.

The experiments showed that, under our experimental conditions, the ovulation of the frogs was not surpressed by the irradiation, and was not changed quantitatively: in all three combinations in which irradiated female-recipients took part, or in which pituitary extract was taken from irradiated frogs (in order to induce ovulation), the rate of the ovulation process and the quantity of ovulating eggs were the same as in the control.

The same results were obtained in series II of the experiments, in which the ovulation process was induced 12 days after the irradiation.

The eggs which were ovulating under these artificial conditions were suitable for experiments with fertilization. For this purpose, the uterine section of the oviduct was opened, the eggs were removed from it, and they were moistened with water containing spermatozoids (the latter was added by mixing seminal fluid in water). It was easy to determine that the eggs were fertilized by judging the rate at which the blastodisc turned upward; this occurred much more rapidly for fertilized eggs than for unfertilized ones.

The artificial fertilization was accomplished in the following ways.

Group I (control). Eggs from an unexposed female whose ovulation was induced by an injection of a suspension of pituitrin from nonirradiated females; spermatozoids from an unexposed male.

Group II. Eggs the same, spermatozoids from an irradiated male.

Group III. Eggs from an irradiated female, the ovulation of which was due to an injection of a pituitrin suspension from nonirradiated females; spermatozoids from an irradiated male. /390

Group IV. Eggs the same, spermatozoids from an unexposed male.

Group V. Eggs from an unexposed female, the ovulation of which was induced by injecting a suspension of pituitary extract from irradiated females; spermatozoids from a nonirradiated male.

Group VI. Eggs the same, spermatozoids from an irradiated male.

Group VII. Eggs from an irradiated female, the ovulation of which was induced by injection of a pituitrin suspension from irradiated females; spermatozoids from an irradiated male.

Group VIII. Eggs the same, spermatozoids from an unexposed male.

The experiments with fertilization on the 5th and 16th days after the irradiation showed that it occurred normally in all these combinations and on both days. In all cases, there was almost 100% fertilization of the eggs (see Table 1). Thus, the fertilization

process, just as the ovulation process, was not disordered by the effect of ionizing radiation in the specified doses. However, this situation did change subsequently.

As we can see in Table 1, in the control group (I), 87% of the eggs reached a stage of 4 blastomeres, 81% reached the stage of neurala, 79% reached the stage of the first movements of the embryo in the form of lateral curvatures of the body into ring-shape, and 75% reached the stage of free floating.

TABLE 1

SURVIVAL RATE OF THE EMBRYOS (%)

Group	Fertilization On The 5th Day After Irradiation				Fertilization On The 16th Day After Irradiation			
	To Stage Of 4 Blastomeres	To Stage Of Neurula	To Stage Of Lateral Movements Into A Ring	To Free Floating Stage	To Stage Of 4 Blastomeres	To Stage Of Neurula	To Stage Of Lateral Movements Into A Ring	To Free Floating Stage
I	87	81	79	75	96	90	89	89
II	56	14	6	0	86	7	0	—
III	58	7	4	0	48	0	—	—
IV	65	55	52	34	58	15	0	—
V	76	63	56	38	66	19	0	—
VI	61	6	2	0	45	1	0	—
VII	38	5	0	—	35	0	—	—
VIII	66	63	50	34	42	19	0	—

In Group IV, in which the female was subjected to irradiation before inducement of ovulation, but the ovulation process was due to the pituitrin taken from normal females and the fertilization was accomplished with normal spermatozoids, the survival rate was much lower. In this case, 65% reached the stage of 4 blastomeres, 55% reached neurula, 52% reached the stage of movements into a ring, and 34% of the embryos reached the stage of free floating.

In Group II, where the female was normal and the ovulation was induced by normal pituitrin, but spermatozoids which were taken from irradiated males took part in the fertilization of the normal eggs, none of the embryos reached the stage of free floating. Fifty-six percent passed through the stage of 4 blastomeres, thirteen percent passed through the neurula stage, and only six percent

of the embryos passed through the stage of movements into a ring.

Even lower results were obtained in those cases in which the fertilization occurred on the 16th day after the irradiation of the animals. Even in Group IV, the developmental process was arrested at the neurula stage, and only 15% of the eggs reached that stage; in Group II, only 7% reached the neurula stage.

In Group V (in which the ovulation of normal females was induced by pituitrin taken from irradiated females on the 12th day after irradiation, while the fertilization was accomplished with normal spermatozoids), the developmental process was also arrested at the neurula stage, while only 19% of the eggs reached that stage. Only 66% of the eggs, in contrast to 96% in the control group, reached the stage of 4 blastomeres. This dependence of the embryonic development on what type of pituitrin induced ovulation was also seen (less sharply, but still rather clearly) in the case when the ovulation was induced on the day following the irradiation of the pituitary body. /391

The effect of the disorder in the function of the pituitary body on the embryogeny was also found in other groups, as can be seen by comparing Group II to Group VI, or Group III to Group VII: in those cases when the ovulation was induced by pituitrin taken from irradiated females, under otherwise equal conditions, the embryogeny was much more disordered.

The greatest disorders in the embryogeny process occurred in Group VIII, in which spermatozoids and eggs of irradiated frogs were used, while the ovulation was induced by injecting pituitrin taken from irradiated frogs.

The data presented show that serious disorders in embryogeny are found in the case of irradiating both males and females. However, when both males and the females are subjected to equal doses of radiation, the pernicious afteraffects are found to a greater degree and at earlier periods in the females than in the males.

Since the spermatozoids were mature at the moment of the irradiation, we can assume that the penetrating radiation had a direct injurious effect on them. Under our experimental conditions, the eggs were still not ready for fertilization at the moment of the irradiation; their maturation occurred after the irradiation. In this case, the injuries to the functions of the maternal organism had particular significance.

Without disregarding the direct injurious effect of the penetrating radiation on the eggs (which is indicated by the fact of the disorders in the embryogeny for the combination in which an irradiated female took part, while the pituitrin which caused the ovulation was normal), we must acknowledge that the injuries to the function of the pituitary body played a substantial role.

This follows from the fact that pituitary bodies from irradiated frogs, removed in order to induce ovulation, are sufficient for a disorder in the embryogeny process. In this respect, although the cells were not subjected to the effect of penetrating radiation, they were nevertheless defective, since their ovulation was induced by irradiated pituitary bodies.

Consequently, it is not only the quantitative aspect of the ovulation process which is linked with the function of the pituitary body under normal conditions, i.e., the rate of the process and the quantity of ovulating eggs; the qualitative aspect is also affected. The quantitative aspect is not disordered by the irradiation (in the dose we used), while the qualitative aspect suffers to a very great degree. Therefore, we must acknowledge the fact that one of the mechanisms which disorder embryonic development, even when it occurs outside of the maternal organism, is the injury to the function of the mother's pituitary gland caused by penetrating radiation.

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